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Contributed Paper

Determining the Optimum Proportions of Cassava Starch Wastewater, Hydrogenic Effluent and Anaerobic Sludge for Methane Production

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ABSTRACT

The optimum proportions of cassava starch wastewater, hydrogenic effluent and anaerobic sludge for batch methane production were determined using D-optimal mixture design. The optimum percentage ratio of cassava starch wastewater: hydrogenic effluent: anaerobic sludge was 7.60 : 16.67 : 75.73 at volatile solid (VS) of 60 g/L which resulted in the highest methane yield (MY) of 108.3 mL CH_4/g-VS. Enhancement of methane production was attempted by supplementing with modified basal medium and modified basic anaerobic (BA) medium. Addition of modified BA medium enhanced MY by 8.28% (113.7 mL CH_4/g-VS) in comparison to a control (without media addition). However, no improvement in MY was observed when using modified basal medium. Replacement of yeast extract by soybean and fish meal at the same total nitrogen concentration in modified BA medium, the differences on MY were only 3.58 % and 4.36% , respectively, indicating that soybean and fish meal can be used as cheaper alternative organic nitrogen sources for methane production.

Keywords: anaerobic digestion, renewable energy, biogas, industrial waste, co-digestion, mixture design

1. INTRODUCTION

Biogas generated by anaerobic digestion (AD) composes mainly of hydrogen and methane. Hydrogen is produced from acidogenesis stage by hydrogen producing bacteria. Only 7.5-15% of energy in organic materials is converted to hydrogen by microbial fermentation at the end of acidogenesis stage [1]. Approximately 65% of remaining energy was in the acidogenesis products, which include volatile fatty acid (VFAs) such as lactic, acetic and butyric acids and alcohols such as ethanol and butanol [1,2]. VFAs can be further used as substrates to produce methane by acetogenic and methanogenic bacteria in the methanogenesis stage of AD.

Level of VFAs is the most important factor for controlling AD process. VFAs buildup is the result of unbalanced digestion process due to a drop in pH following high VFAs production. The hydrogenic effluent is the waste obtained after hydrogen production process. It still has a high chemical oxygen demand (COD) concentration containing VFAs and alcohol which can be further used as substrate to produce methane. Therefore, the utilization of hydrogenic effluent as co-substrate for methane production not only reduce load of COD to the environment or a cost associated with its disposal, but also energy can be attained. The effluent from hydrogen production in acidogenesis stage, affects methanogenesis stage by lowering the activity of methanogens [3]. Buswell et al. [4] had reported that toxic conditions resulting from VFAs could be reduced by lowering the organic loading or by dilution. In this study, cassava starch wastewater was used as a co-substrate of hydrogenic effluent in order to reduce the toxic environment resulting from high concentration of VFAs in the effluent.

Cassava starch wastewater contains high carbohydrates, organic matters and high COD content representing an energy-rich resource, which can be potentially converted to a wide variety of useful products including methane. Its utilization in methane production was not only a way to reduce its heavy load caused by disposal to the environment or high cost associated with its disposal, but also beneficial since bioenergy was produced [5]. Numbers of researchers have investigated the use of cassava starch wastewater for biogas production. For example, Intanoo et al. [3] studied the effects of COD loading rate on hydrogen production from cassava starch wastewater in an upflow anaerobic sludge blanket (UASB) bioreactor. The results showed that the hydrogen yield (HY) increased from 15.82 to 39.83 L H_2/kg-$COD_{removed}$ when COD loading rate was increased from 10 to 25 kg/m^3.d. High COD loading rate provided higher substrate concentration for hydrogen producing bacteria. Further increase in COD loading rate from 25 to 30 kg/m^3.d resulted in a decrease in hydrogen production due to higher VFAs concentration when increasing COD loading rate and limited VFAs tolerance in hydrogen producing bacteria.

Although high carbon content in cassava starch wastewater provides good potential in biogas formation, it causes acid forming bacteria to quickly produce VFAs resulting in a significant pH drop to a value that could cause inhibition on growth of methanogens [3]. In this study, anaerobic sludge was introduced in order to alleviate this problem. Anaerobic sludge is a by-product from wastewater treatment system. It contains high nitrogen contents and various types of microorganisms, making it suitable as an additional co-substrate and also an inoculum in biogas production [6]. By mixing anaerobic sludge with cassava starch wastewater and hydrogenic effluent in methane production, it would be expected that microorganisms in anaerobic sludge would immediately consume VFAs while it also served as nitrogen source in the production.

Mixture design is a statistical method used to generate suitable proportions of components in a mixture. Typical trial and error approach requires higher cost and longer time to obtain good mixture proportions. In recent years, mixture design has been used to optimize proportions of substrate for methane production. Proportions of dairy manure, chicken manure and rice straw were optimized for methane production using mixture design [7]. The maximum methane yield (MY) of 336 mL CH_4/g-volatile solid (VS) was obtained from dairy manure: chicken manure: rice straw proportions of

44.50: 35.00: 20.50. The results indicate that synergistic effect of microbial activities and more balanced chemical compositions was observed when using co-substrate for methane production.

In this research, cassava starch wastewater, hydrogenic effluent and anaerobic sludge were used as substrates and inoculum in methane production. Proportions of the three components were optimized using D-optimal mixture design with the aim to maximize MY as response. Enhancement of MY was investigated by supplementing different media, i.e., modified basal and modified basic anaerobic (BA) media, to the substrates at the optimum proportions. In addition, replacement of yeast extract by soybean, Aji-L (liquid waste from monosodium glutamate production) and fish meal were also carried out in order to search for a low-cost alternative nitrogen source.

2. MATERIALS AND METHODS

2.1 Feedstocks

Cassava starch wastewater was obtained from National Starch and Chemical Co. Ltd., Kalasin, Thailand. Hydrogenic effluent was the effluent from hydrogen production by a co-digestion of cassava starch wastewater and buffalo dung in a 6-L continuous stirred tank reactor carried out in our laboratory. Anaerobic sludge was used as nitrogen source and inoculum. It was collected from an internally circulated wastewater treatment facility of Khon Kaen Brewery Co. Ltd., Khon Kaen, Thailand. All materials were stored at 4 °C before use. Total solid (TS) content of the cassava starch wastewater, hydrogenic effluent and anaerobic sludge were 25.18 g/L, 14.18 g/L and 133.05 g/kg, respectively. VS contents of the three feedstocks were 11.03 g/L, 17.10 g/L and 120 g/kg in respective order.

Soybean and fish meal were collected from Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. Aji-L was obtained from Ajinomoto (Thailand) Co., Ltd., Pathum Thani, Thailand. Particle sizes of soybean and fish meal were reduced by food blender and filtered through a 2-mm screen to remove any large particles. Total nitrogen (TN) contents of soybean, fish meal and Aji-L, determined by Kjeldahl method [8] were 7.7, 8.9 and 3.44 %, respectively. In addition, NH_4Cl in modified BA medium was replaced by $(NH_4)_2SO_4$ as an inorganic nitrogen source.

2.2 Medium

The modified basal medium (modified from Cuzin et al. [9]) and modified BA medium (Fangkum and Reungsang [10] modified from Angelidaki and Sanders [11]) were used in the enhancement of methane production. The stock solution of modified basal medium and modified BA medium and amounts of use were showed in Table 1 and 2, respectively.

Table 1. Stock solution of modified basal medium [9].

Nutrient (g/L)	Usage (mL/L)
(A) K_2HPO_4, 125	2
(B) $NaHCO_3$, 50	50
(C) NH_4Cl, 30; NaCl, 100; $MgCl_2.6H_2O$, 40; $CaCl_2.2H_2O$, 16; KCl, 50	10
(D) CH_3COONa, 250; NaS_2O_4, 100; Na_2S, 250; H_3BO_3, 6; $CuCl_2.2H_2O$, 2; Na_2SeO_3, 0.173	1
(E) Yeast extract, 1000	1

Table 2. Stock solution of modified BA medium [10]

Nutrient (g/L)	Usage (mL/L)
(A) $K_2HPO_4.3H_2O$, 200	2
(B) $NaHCO_{3,}$ 52	50
(C) NH_4Cl, 100; NaCl, 10; $MgCl_2.6H_2O$, 10; $CaCl_2.2H_2O$, 5;	10
(D) $MnCl_2.4H_2O$, 0.05; $AlCl_3$, 0.05; $NiCl.6H_2O$, 0.092; $FeCl_2.4H_2O$,2; $CuCl_2.2H_2O$, 0.038; H_3BO_3, 0.05; $(NH_4)_6Mo_7O_{24}.4H_2O$, 0.05; $ZnCl_2$, 0.05; $CoCl_2.6H_2O$, 0.05, $Na_2SeO_3.5H_2O$, 0.1, concentrate HCl, 1 mL; ethylenediaminetetraacetate, 0.5	1
(E) Yeast extract, 100	1

2.3 Determination of Optimum Proportions of Feedstocks Using a Mixture Design

D-optimal mixture design was used to design experimental runs in order to determine the optimum proportions of cassava starch wastewater (X_1), hydrogenic effluent (X_2) and anaerobic sludge (X_3) that maximized the MY. Mixtures were designed with varying proportions of materials at a total VS concentration of 60 g/L (Table 3). Each component was expressed percentages and the sum of all components (X_1+X_2+X_3) was added up to 100. Nineteen experimental points were designed using Design-Expert software (Demo version 7.0, Stat-Ease, Inc., Minneapolis, MN, USA).

Table 3. Mixture experimental design defining proportions of cassava starch wastewater, hydrogenic effluent and anaerobic sludge and respective values of MY.

Run	Experimental factors			MY (mL CH_4/g-VS)	
	Cassava starch wastewater (%, actual)	Hydrogenic effluent (%, actual)	Anaerobic sludge (%, actual)	Observed[a]	Predicted
1	0.00	16.70	83.30	97.1 ± 1.86	92.5
2	13.40	0.00	86.60	85.1 ± 1.21	85.4
3	11.50	10.60	77.90	104.2 ± 2.12	98.8
4	7.10	1.30	91.70	74.1 ± 1.05	66.5
5	16.70	8.20	75.10	93.7 ± 1.40	91.0
6	9.30	15.70	75.00	107.8 ± 1.06	106.3
7	5.40	14.20	80.30	95.3 ± 1.15	100.8
8	14.10	4.80	81.20	95.1 ± 1.68	92.5
9	1.00	7.40	91.70	51.5 ± 1.22	56.4
10	16.70	8.20	75.10	88.2 ± 1.82	91.0
11	9.30	15.70	75.00	101.8 ± 0.67	106.3
12	8.20	8.80	83.00	87.3 ± 0.95	92.6
13	0.00	16.70	83.30	94.0 ± 1.80	92.5
14	2.90	10.80	86.30	79.3 ± 0.21	81.6
15	8.30	8.30	83.30	96.2 ± 1.65	91.8

Table 3. Continued.

Run	Experimental factors			MY (mL CH_4/g-VS)	
	Cassava starch wastewater (%, actual)	Hydrogenic effluent (%, actual)	Anaerobic sludge (%, actual)	Observed[a]	Predicted
16	8.30	8.30	83.30	91.3 ± 0.91	91.8
17	1.00	7.40	91.70	56.4 ± 1.64	56.4
18	13.40	0.00	86.60	78.7 ± 1.77	85.4
19	8.30	8.30	83.30	94.4 ± 1.59	91.8

[a] The values of observed MY were obtained from triplicate samples.

Different models including linear (Eq.1), quadratic (Eq.2) and cubic models (Eq.3) were used to analyze the mixture proportions that resulted in the maximum MY. Equations of each model used in the analysis are as follows:

$$Y = \sum_{i=1}^{p} \beta_i x_i \quad (1)$$

$$Y = \sum_{i=1}^{p} \beta_i x_i + \sum\sum_{i<j}^{p} \beta_{ij} x_i x_j \quad (2)$$

$$Y = \sum_{i=1}^{p} \beta_i x_i + \sum\sum_{i<j}^{p} \beta_{ij} x_i x_j + \sum\sum_{i<j}^{p} \delta_{ij} x_i x_j (x_j - x_i) + \sum\sum\sum_{i<j<k}^{p} \beta_{ijk} x_i x_j x_k \quad (3)$$

From the above equations, Y is MY; β_i, β_{ij}, β_{ijk} are linear, quadratic and cubic coefficients and δ_{ij} is a parameter of the model. The term $\beta_i x_i$ represents linear mixing proportion and the parameter β_{ij} represents synergistic or antagonistic effect from proportions mixing.

2.4 Methane Production

Batch experiments were conducted in 120 mL serum bottles containing cassava starch wastewater, hydrogenic effluent and anaerobic sludge according to the experimental design (Table 3). Distilled water was supplied to the bottles for making up a working volume to 80 mL. Initial pH was adjusted to 7.5 using 5 N NaOH or 5 N HCl. Liquid in the bottle was flushed with pure nitrogen gas for 5 minutes in order to create anaerobic condition. The bottle was then sealed with a rubber stopper and an aluminum cap. The head space was then flushed with nitrogen gas for 5 minutes to remove oxygen and to create anaerobic condition. The bottles were incubated at room temperature (30 ± 2 °C) on an orbital shaker at 150 rpm. During the fermentation, total biogas volume was determined using a wetted glass syringe method [12].

For enhancement of MY, modified basal medium and modified BA medium were compared for their abilities to act as enhancing media. Methane production were conducted in 120 mL serum bottles containing cassava starch wastewater, hydrogenic effluent and anaerobic sludge at the optimum proportions. The stock solutions of modified basal medium or modified BA medium were added to the bottles at the amounts indicated in table 1 and 2, respectively. The working volume was then adjusted to 80 mL by distilled water. Control set was methane fermentation under the optimum proportions without medium addition.

As the results indicated that MY was mostly enhanced by modified BA medium, the further experiments were conducted to investigate the effect of replacing yeast extract in the modified BA medium by low cost organic nitrogen sources on methane production. Yeast extract contained in modified BA medium was replaced by soybean, fish meal and Aji-L at the same TN concentration (9.50%). The aim of the replacement of yeast extract in BA medium by soybean, Aji-L and fish meal was to search for the alternative nitrogen source with a high nitrogen content but has a low cost or no cost. Soybean and Aji-L are the wastes obtained from the production process of soybean oil and monosodium glutamate, respectively. Fish meal is a fish product that is intended not to use for human consumption. In addition, the effect of inorganic nitrogen replacement was investigated by replacing NH_4Cl in the modified BA medium by $(NH_4)_2SO_4$ at the same TN concentration of 26.17%.

2.5 Analytical Methods

Physical properties including TS and VS were determined using standard methods [13]. TN was analyzed by Kjeldahl method [8]. Total sugar concentration was measured by phenol sulfuric acid method with glucose as a standard [14]. The pH was measured using a pH meter (pH 500 Clean, USA).

Concentrations of VFAs and alcohols were analyzed using high performance liquid chromatography (HPLC) (DGU-20A, Shimadzu) with a Vertisep™ OA 8 μm 7H column. HPLC conditions followed the method of Selembo et al. [15]. Biogas compositions were determined using a gas chromatography (GC) (GC 2014, Shimadzu) equipped with a thermal conductivity detector (TCD) and a 2-m stainless steel column packed with Shin carbon (50/80 mesh). GC conditions followed the method of Laocharoen et al. [16].

3. Results and Discussion

3.1 Model Fitting and Response Variables Analysis

By varying proportions of cassava starch wastewater, hydrogenic effluent and anaerobic sludge following D-optimal mixture design, the results were demonstrated in Table 3.The analysis of variance (ANOVA) showed that all models were significant (p-value < 0.0001) (Table 4). However, R^2 value of the linear model was much lower than quadratic and cubic models indicating an inadequate fitting of the linear model with experimental data [17]. Both cubic and quadratic models were tested for the lack of fit. The p-values of 0.1794 of cubic model and 0.0735 of quadratic model indicated that the lack of fit were not significant in both models at 95% confidence level. These results suggested that both models could be used for prediction [7]. Although R^2 value of cubic model (0.9653) was higher than the value of quadratic model (0.9194), the predicted R^2 value of the quadratic model (0.8031) was higher. Thus, the quadratic model was selected to fit the experimental data. A high predicted R^2 value indicated a close agreement between experimental results and values predicted by the model.

Table 4. ANOVA of different model for methane yield.

Model	*F*-value	*p*-value	R^2	Adjusted R^2	Predicted R^2	*p*-value of Lack of fit
Linear	16.83	0.0001	0.6778	0.6375	0.5054	0.0023
Quadratic	29.67	< 0.0001	0.9194	0.8884	0.8031	0.0735
Cubic	27.85	< 0.0001	0.9653	0.9307	0.6472	0.1794

The quadratic model was calculated from D-optimal design data. ANOVA of the model was presented in Table 5. Model F-value of 29.67 and *p*-value of less than 0.05 indicated that the model was significant at 95% confidence level. R^2 value of 0.9194 and adjusted R^2 value of 0.8884 suggested that the model can be used to explain 89 - 92% variability in the response variable. Additionally, lack of fit of the model was not significant (*p*-value of 0.0735) indicating that the resulting quadratic model can suitably describe the MY. The model was as followed:

$$Y= -1791.70X_1 + 81.75X_2 + 9.85X_3 + 145.76 X_1X_2 + 2732.15X_1X_3 + 509.04X_2X_3 \quad (4)$$

From Eq. (4), Y was MY in mL CH_4/g-VS; X_1, X_2 and X_3 were cassava starch wastewater, hydrogenic effluent and anaerobic sludge, respectively.

Table 5. ANOVA for the quadratic model regression representing methane yield in mixture design.

Source	Sum of squares	df	Mean squares	*F*-Value	*p*- value Prob > *F*
Model	3604.02	5	720.8	29.67	<0.0001
Linear mixture	2656.87	2	1328.43	54.67	<0.0001
X_1X_2	1.59	1	1.59	0.066	0.8019
X_1X_3	713.58	1	713.58	29.37	0.0001
X_2X_3	19.76	1	19.76	0.81	0.3836
Residual	315.87	13	24.30		
Lack of fit	232.70	6	38.78	3.26	0.0735
Pure error	83.16	7	11.88		
Cor total	3919.89	18			
R^2	0.9194				
Adjusted R^2	0.8884				

X_1: Cassava starch wastewater, X_2: Hydrogenic effluent, X_3: Anaerobic sludge.

Based on the model given in Eq. (4), maximum MY of 108.3 CH_4/g-VS was predicted at cassava starch wastewater of 7.60%, hydrogenic effluent of 16.67% and anaerobic sludge of 75.73%. A three dimensional response surface plot (Figure 1) based on Eq. (4) was constructed to determine the effects of components and their interactions on MY. The plot indicated that mixture of co-substrates increased methane production indicating the synergetic effects within the system. Optimum proportions positively influenced methane production by optimizing carbon and nitrogen concentrations, contributing to buffering capacity and balancing macro and micronutrients of the system [18].

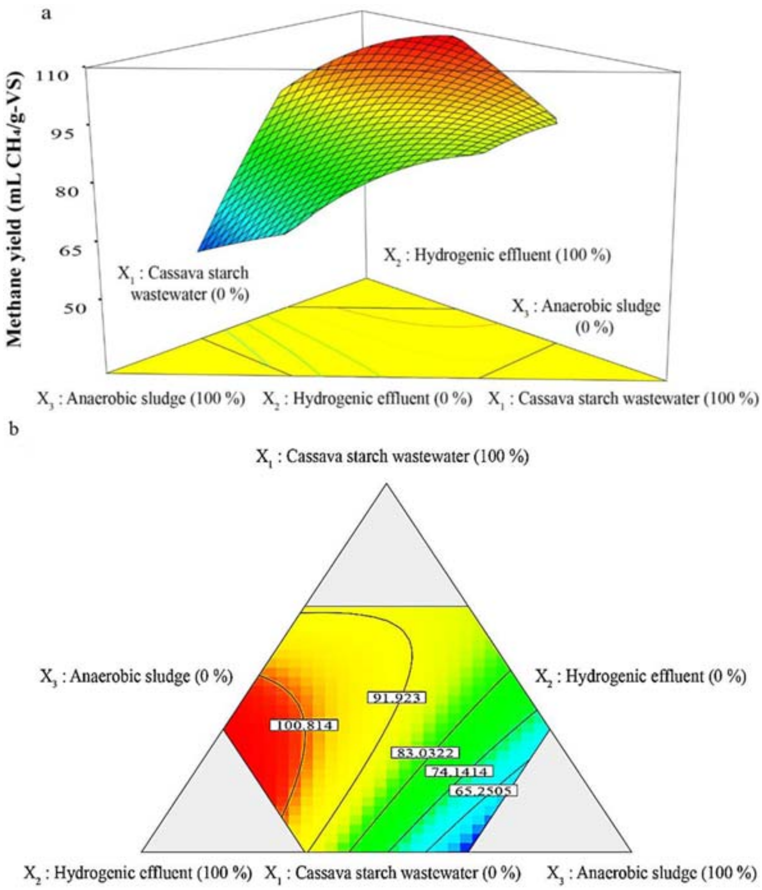

Figure 1. The surface (a) and contour plot (b) of mixture design for methane yield: X_1; cassava starch wastewater, X_2; hydrogenic effluent and X_3; anaerobic sludge.

3.2 Synergistic and Antagonistic Effects

Co-digestion of substrates can exhibit synergistic or antagonistic effects. A higher MY in co-digestion when compared to individual substrate fermentation indicates synergistic effect. In contrast, co-digestion that resulted in lower MY when compared to individual substrate fermentation indicates antagonistic effect. Synergistic and antagonistic effect on MY from mixture of cassava starch wastewater, hydrogenic effluent and anaerobic sludge was calculated by dividing experimental MY by calculated MY (Table 6). The ratio higher than 1 indicates synergistic effect of mixture proportions while the ratio lower than 1 indicates antagonistic effect of mixture proportions. The experimental MY was obtained from yield of methane produced by each proportions from experimental design while the calculated MY was obtained from the MY of the sole substrates based on the VS of each substrate contained in the mixture [19]. MY of 28.8, 64.9 and 20.3 mL CH_4/g-VS were obtained when cassava starch wastewater, hydrogenic effluent and anaerobic sludge were used as a sole substrate (control experiments), respectively (Table 6). Experimental MY/calculated MY ratio of 3.70 was obtained under the optimal proportions suggesting synergistic effect and indicated the suitable proportions of cassava starch wastewater, hydrogenic effluent and anaerobic sludge as substrates for methane production.

Table 6. Synergistic and antagonistic effects of co-digestion proportions on MY.

Run	Experimental MY ($mL\ CH_4$/g-VS)[a]	Calculated MY ($mL\ CH_4$/g-VS)	Experimental MY Calculated MY ratio*
Cassava starch wastewater	28.8 ± 0.38	28.8	-
Hydrogenic effluent	64.9 ± 0.51	64.9	-
Anaerobic sludge	20.3 ± 0.22	20.3	-
1	97.1 ± 1.86	27.7	3.51
2	85.1 ± 1.21	21.4	3.98
3	104.2 ± 2.12	26.0	4.01
4	74.1 ± 1.05	21.5	3.45
5	93.7 ± 1.40	25.4	3.69
6	107.8 ± 1.06	28.1	3.84
7	95.3 ± 1.15	27.1	3.52
8	95.1 ± 1.68	23.7	4.01
9	51.5 ± 1.22	23.7	2.17
10	88.2 ± 1.82	25.4	3.47
11	101.8 ± 0.67	28.1	3.62
12	87.3 ± 0.95	24.9	3.51
13	94.0 ± 1.80	27.7	3.39
14	79.3 ± 0.21	25.4	3.12
15	96.2 ± 1.65	24.7	3.90
16	91.3 ± 0.91	24.7	3.70
17	56.4 ± 1.64	23.7	2.38
18	78.7 ± 1.77	21.4	3.68
19	94.4 ± 1.59	24.7	3.82
Optimum proportions	105 ± 1.63	28.4	3.70

[a] The values of experimental MY were obtained from triplicate samples

*Experimental MY/ Calculated MY ratio <1; Antagonistic effect

*Experimental MY/ Calculated MY ratio =1; The substrate work independently from the mixture

*Experimental MY/ Calculated MY ratio >1; Synergistic effect

Different substrates have different group of indigenous microorganisms and nutrient compositions. It is expected that cassava starch wastewater contains low amount of methanogens with excess amount of organic matters, which resulted in low rate of methane production by self-fermentation. Addition of hydrogenic effluent, which mainly contains acidogenic bacteria, can speed up conversion of organic matters in the cassava starch wastewater to VFAs. These combinations can improve the methane production since VFAs produced are readily consumed by methanogens which are the dominant microorganisms in the anaerobic sludge. Suitable proportions of substrates could provide appropriate balance of nutrients in term of C:N:P ratio which could improve methane production efficiency in comparison to single substrate fermentation.

In this study, synergistic effect on MY was found in every treatments (run 1 - 19) indicated by the experimental MY/ calculated MY ratio were in the ranges of 2.17 - 4.01. Our results implied that a co-digestion of cassava starch wastewater, hydrogenic effluent and anaerobic sludge has a synergism effect on MY.

3.3 Model Validation

From the optimization plots (Figure 1), the predicted MY of 108.3 mL CH_4/g-VS was obtained from the optimum mixture proportions of cassava starch wastewater: hydrogenic effluent: anaerobic sludge at 7.60: 16.67: 75.73 (%). In order to validate the model, batch experiment was conducted using optimum proportions of the co-substrates. The MY of 105 mL CH_4/g-VS was obtained from confirmation experiment. This value was only 3.05% different from predicted MY. The result indicated that the model is valid and can be used to optimize the proportions of co-substrates for methane production.

3.4 Comparison of Methane Production to Previously Reported Values

MY obtained in this study was compared with previous reports that employed mixture design to optimize the proportions of substrates for methane production. Reported MY in the literature were varied in the range of 207-764.9 mL CH_4/g-VS (Table 7). The highest MY of 764.9 mL CH_4/g-VS was obtained from a co-digestion of solid cattle slaughterhouse waste, manure, various crops and municipal solid wastes under thermophilic conditions to produce methane [18]. MY of 473.2 mL CH_4/g-VS was obtained from the ratio of sewage sludge, cow dung and garden waste at 70: 20.29: 9.71% while a lower MY of 442.2 mL CH_4/g-VS was attained when sewage sludge was mixed with cow dung at 75.5: 24.5% without addition of fruit juice [20]. Results indicated that an addition of garden waste improved MY by only 7% which implied that main substrates for methane production were sewage sludge and cow dung. High organic content and various microbes in sewage sludge and cow dung might be suitable for methane production. Our results showed the optimum proportions of cassava starch wastewater, hydrogenic effluent and anaerobic sludge for methane production at 7.60: 16.67: 75.73% at a VS of 60 g/L. Highest proportion of anaerobic sludge indicated that it acted as the inoculum source as well as the main substrate for methane production. Differences in MY depended on types and concentrations of substrates, inoculum and fermentation conditions.

3.5 Enhancement of Methane Production

Addition of modified basal medium and modified BA medium to the optimum proportions of cassava starch wastewater, hydrogenic effluent and anaerobic sludge were investigated for a possible enhancement in MY. Figure 2 showed time course profiles of methane production, total sugar and VFAs consumption rate when modified basal medium and modified BA medium were added (Figure 2a-b). The lowest cumulative methane production (CMP) of 6,103 mL CH_4/L and MY of 101.7 CH_4/g-VS were obtained when using modified basal medium (Figure 2a). This values were lower than the control experiment which resulted in CMP and MY of 6,299 mL CH_4/L and 105 mL CH_4/g-VS, respectively (Figure 2c). In contrast, an addition of modified BA medium showed maximum CMP and MY of 6,822 mL CH_4/L and 113.7 CH_4/g-VS, respectively (Figure 2b). These results were 8.29% greater than the control experiment.

Table 7. Comparisons of methane yield from various mixtures of biomass using mixture design.

Substrate	Design	Optimum proportions (%)	Methane yield (mL CH_4/g-VS)	Ref
Dairy manure + chicken manure + rice straw	simplex-centroid	44.50 : 35.00 : 20.50	336	[7]
Solid cattle slaughterhouse waste + manure + various crops + municipal solid wastes	simplex-centroid	35.41 : 0 : 0.46 : 64.13	764.9	[18]
Garden waste + cow dung + sewage sludge	simplex-centroid	9.71 : 20.29 : 70.00	473.2	[20]
Sewage sludge + cow dung +fruit juice	simplex-centroid	75.50 : 24.50 : 0.00	442.4	[20]
paper industry sludge + chemical industry sludge + petrochemical industry sludge + automobile industry sludge + food processing industry sludge	simplex-centroid	0 : 100 : 0 : 0 : 0	207	[21]
Cassava starch wastewater + hydrogenogenic effluent + anaerobic digestion sludge	D-optimal	7.60 : 16.67 : 75.73	108.3	This study

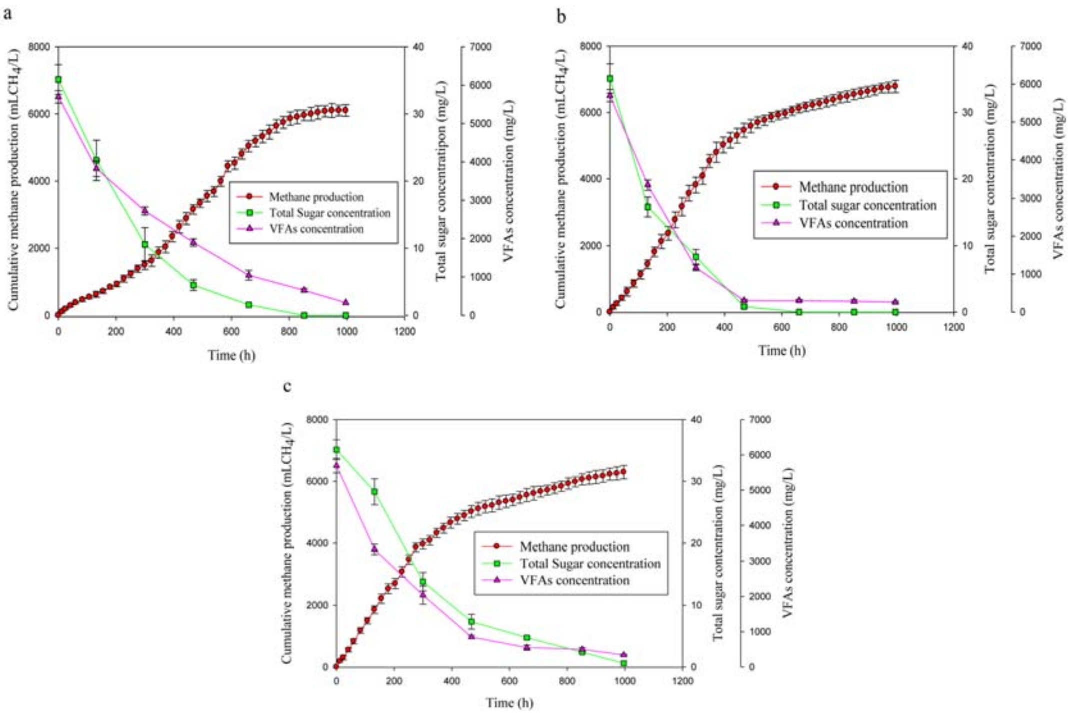

Figure 2. Effect of medium addition on methane production, total sugar and VFAs consumption at the optimum cassava starch wastewater, hydrogenic effluent and anaerobic sludge of 7.60: 16.67: 75.73%. (a) modified basal medium, (b) modified BA medium, (c) control (without medium).

The results suggested that trace elements contained in modified basal and modified BA medium affected CMP and MY. Trace elements positively affect the metalloenzymes involved in metabolism of methanogens or in methanogenesis process [22]. Modified basal medium contained only Cu, whilst, modified BA medium contained more kinds of trace elements such as Co, Cu, Ni, Fe, Zn, Mo and Mn. Therefore, with an addition of modified BA medium, CMP and MY were enhanced. Feng et al. [22] reported that Ni, Mo, B showed positive effects on archaea population and *Methanosarcinar* sp. resulting in an increase of methane from 7 to 15%. In addition, Zhang et al. [23] found that trace elements increased VFAs degradation rate and MY from co-substrate fermentation while Ca and Mg salts added as energy supplement could prevent foaming and improve the MY.

It is also interesting to observe that a modified BA medium contained a lower concentration of yeast extract (100 mg/L) in comparison to modified basal medium (1,000 mg/L). A high concentration of yeast extract released ammonia which was toxic to methanogens [24]. Yeast extract contains amino acid, peptide, vitamin B complex, trace elements (i.e., Cu, Fe, Mg, Zn, Ni and V) [25]. Some vitamins in yeast extract are co-factors of enzymes involved in microorganisms metabolic process.

Addition of both modified BA and modified basal media resulted in higher total sugar consumption rate when compared with the control, with the highest rate of 1.25 mg/L.d occurred with the addition of modified BA medium (Figure 2b). Slightly lower sugar consumption rate of 1.00 mg/L.d was resulted from the addition of modified basal medium (Figure 2a). The control experiment had a lowest sugar consumption rate of 0.84 mg/L.d (Figure 2c). Meanwhile, VFAs consumption rates of the control experiment and addition of modified basal medium were not much different i.e., 127.50 and 127.76 mg/L.d, respectively.

3.6 Effects of Inorganic and Organic Nitrogen Replacement on Methane Production

Yeast extract, an organic nitrogen source in modified BA medium, was replaced by soybean, fish meal or Aji-L at 100 mg/L. For an effect of inorganic nitrogen replacement, NH_4Cl in modified BA medium was replaced by $(NH_4)_2SO_4$. When replacing yeast extract with soybean and fish meal, maximum MY of 112.9 and 112 mL CH_4/g-VS were observed (Figure 3). The differences on MY were only 3.58 % and 4.36 % when compared with MY using yeast extract as organic nitrogen (117.1 mL CH_4/g-VS) under the same TN concentration. Vitamin B complex is an important growth factor for microorganisms. Soybean is a perfect source of vitamin B complex consisting (per 100 g dry weight) of 0.912 mg thiamine, 2.16 mg niacin, 0.523 mg pyridoxine and 0.320 mg riboflavin [26]. Fish meal also rich in vitamins B-complex i.e., cobalamine, niacin, choline, pantothenic acid, and riboflavin [27]. Our results indicated that soybean and fish meal can replace yeast extract as organic nitrogen source. However, lowest MY of 101.3 mL CH_4/g-VS was observed when yeast extract was replaced by Aji-L. The results may due to the inhibitory effect of sulfate contained in Aji-L (4.50 g/L) [28] on methanogens. Similar trend was observed when replacing NH_4Cl with $(NH_4)_2SO_4$ as an inorganic nitrogen source together with addition of soybean, fish meal or Aji-L in which a low MY of 110.8, 108.4 and 99.4 mL CH_4/g-VS were obtained, respectively. Sulfate could be converted to sulfide which is toxic to methanogens. Utilization of high concentration of sulfate resulted in production of sulfide (H_2S) through sulfate reduction [29].

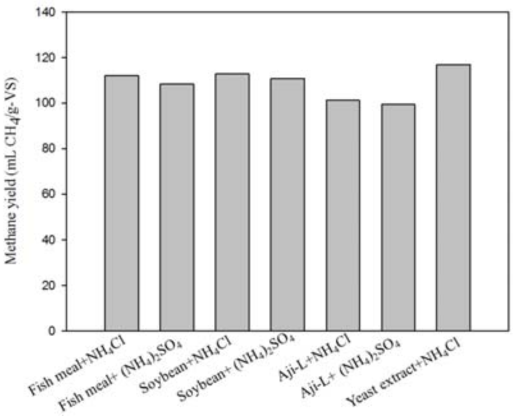

Figure 3. Effect of nitrogen sources replacement on methane yields at the optimum cassava starch wastewater, hydrogenic effluent and anaerobic sludge of 7.60: 16.67: 75.73%.

4. Conclusions

This study successfully demonstrated methane production by co-digestion of cassava starch wastewater, hydrogenic effluent and anaerobic sludge. The optimum proportions of cassava starch wastewater, hydrogenic effluent and anaerobic sludge on methane production were 7.60: 16.67: 75.73 (%) at a VS of 60 g/L. An addition of modified BA medium to the optimum proportions of the co-substrates enhanced MY by 8.28%. Replacement of yeast extract by soybean and fish meal gave a comparable MY to that attained from yeast extract, indicating that soybean and fish meal can be used as organic nitrogen sources in order to reduce capital cost. A replacement of NH_4Cl with $(NH_4)_2SO_4$ did not improve MY suggesting that $(NH_4)_2SO_4$ was inappropriate inorganic nitrogen source for methane production.

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